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Isolation, identification and characterization of microorganisms capable of growing in chocolate pralines

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INTRODUCTION

Chocolate pralines can be spoiled by growth of microorganisms in the filling changing the sensory appearance of the product by production of off-flavors and through crack formation due to gas production. Chocolate praline filling contains 30-50% sugar and up to 10% alcohol, which eliminates growth of most microorganisms. Nonetheless, microorganisms tolerating low water activity have previous been shown to grow in high sugar content food products.

The aim of the present study was to isolate and identify microorganisms, which are present in chocolate pralines and characterize them based on their ability to grow at high sugar and alcohol concentrations.

METHODS

Isolation media: MRS with 40% sucrose, PCA with 40% sucrose, DG18, and MY50G. Incubation at 25°C for 5-28 days.

Identification: Clustering by rep-PCR, GTG_5-primer, 16S and 26S rRNA gene sequencing.

Characterization: MRS broth with 40-70% sucrose and 0-12% ethanol. Growth measured as OD at 460nm.

ISOLATION

Bacteria, yeast and moulds able to grow on sugar rich media were isolated from chocolate pralines.

On media modified with 40% sucrose, counts up to 10^7 CFU/g and 10^5 CFU/g were found in chocolate praline and environmental samples, respectively.

Samples with high CFU/g counts contained mainly yeast. However, bacteria was found in counts up to 10^3 CFU/g.

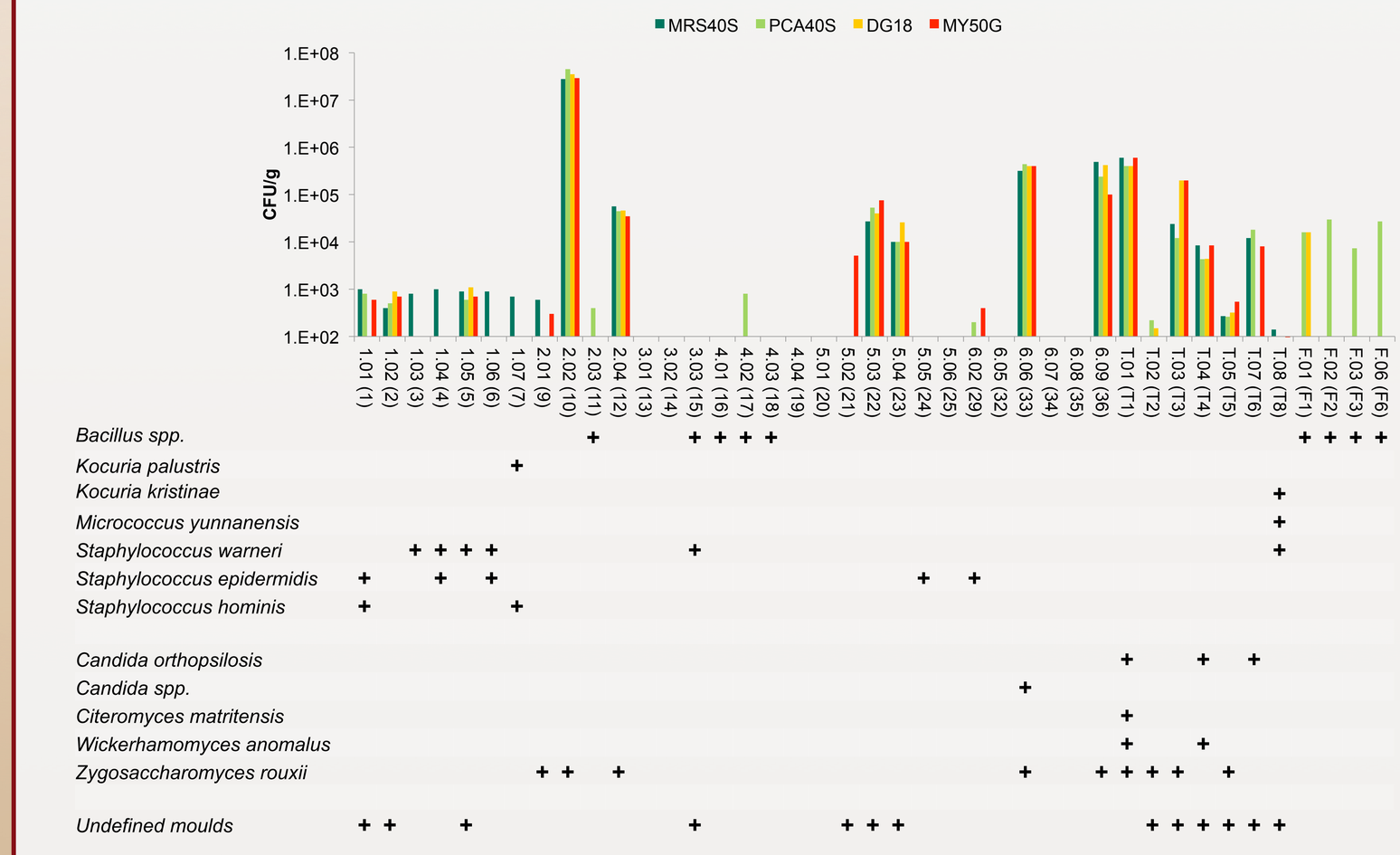

Figure 1: Colony forming units pr. g in chocolate samples determined on MRS40S, PCA40S, DG18, and MY50G media. Samples (1)-(36): small producers, (F1)-(F6): large producer, (T1)-(T8): environmental samples large producer. + : species found in sample.

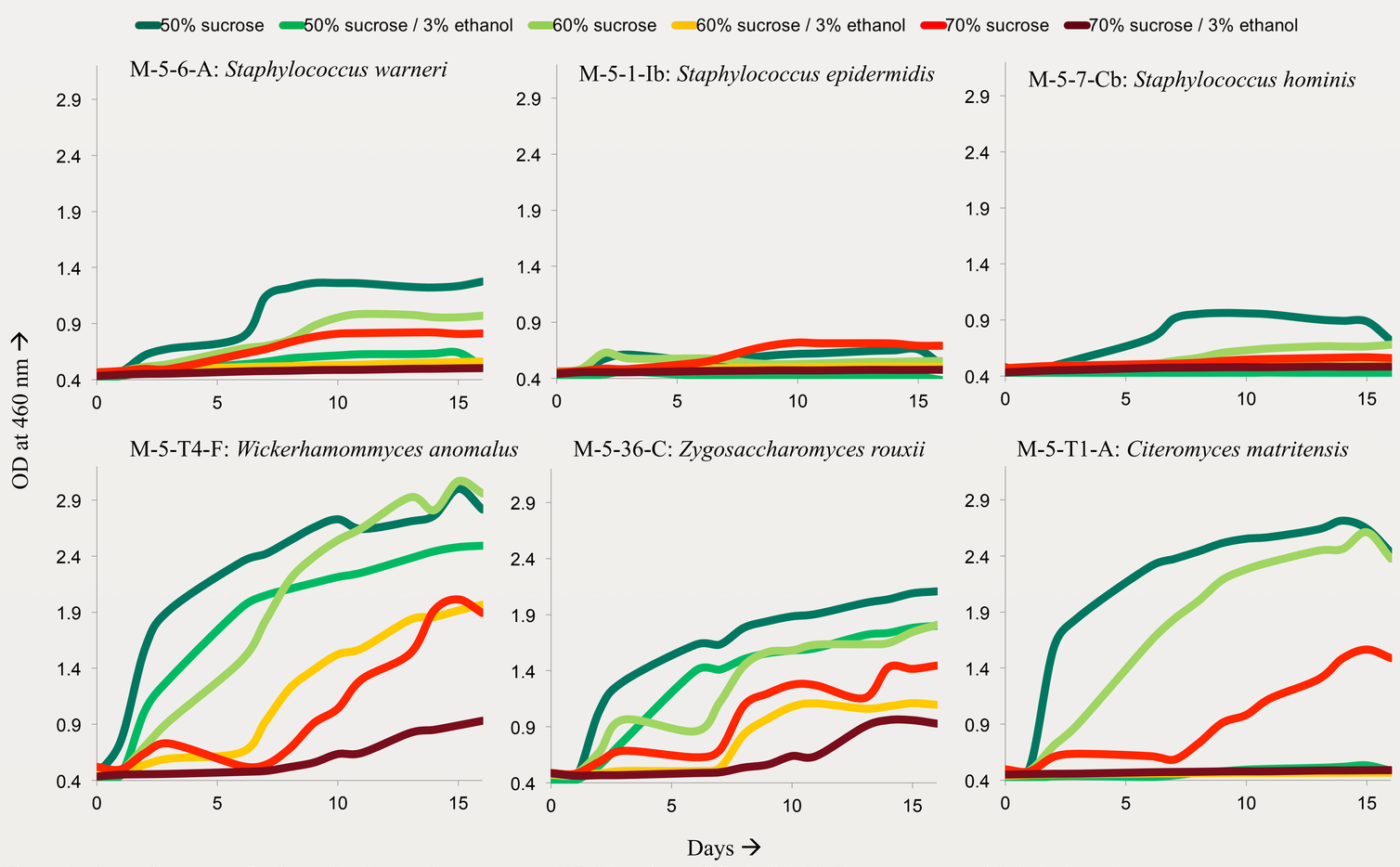

Figure 2: Growth curves of selected isolates when grown in MRS broth modified with 50-70% sucrose and 0-3% ethanol.

IDENTIFICATION

Isolated bacteria belongs to *Staphylococcus* spp. and *Bacillus* spp. mainly. However, also *Kocuria* spp. and *Micrococcus* spp. was found.

Yeasts were identified as belonging to *Wickerhamomyces* spp., *Candida* spp., *Citeromyces* ssp., and *Zygosaccharomyces* spp..

Moulds belong to *Aspergillus* spp. and *Penicillium* spp.

CHARACTERIZATION

All yeast isolates grew at 70% of sucrose. The most sucrose / ethanol tolerant isolate was *Wickerhammyces anomalus.* Growth was observed after two days in 60% sucrose/3% ethanol, and 70/% sucrose/3% ethanol.

The most tolerant bacteria was *Staph. warneri*. Growth was observed after 12 days in 70% sucrose/3% ethanol, and after six days in 40% sucrose/6% ethanol.

Off-flavors, gas, and slime was produced.

CONCLUSION

- On media modified with 40% sucrose, 10^7 CFU/g and 10^5 CFU/g was isolated from chocolate pralines and production environment, respectively.
- *Staphylococcus* spp., *Bacillus* spp., *Zygosaccharomyces* spp., *Candida* spp., *Wickerhamomyces* spp., *Citeromyces* spp., *Aspergillus* spp., and *Penicillium* spp. was isolated.
- *Wickerhamomyces anomalus* and *Zygosaccharomyces rouxii* grow under conditions relevant for spoilage of chocolate pralines.